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# BDNF modifies hippocampal KCC2 and NKCC1 expression in a temporal lobe epilepsy model

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Excitatory GABA actions, induced by altered expression of chloride transporters (KCC2/NKCC1), can contribute to seizure generation in temporal lobe epilepsy. In the present study, we evaluated whether BDNF administration can affect KCC2/NKCC1 expression, ictogenesis and behavioral alterations in this paradigm. Status epilepticus was induced in male rats with pilocarpine, followed by a treatment of either a single high dose or multiple injections of BDNF during the latent phase of temporal lobe epilepsy. Chloride transporters expression, spontaneous recurrent seizures, and hyperexcitability post-seizural behaviors were evaluated after treatment. NKCC1 protein expression was markedly upregulated, whereas that of KCC2 was significantly downregulated in epileptic hippocampi compared to intact controls. Application of BDNF (both single high dose and multiple injections) increased KCC2 expression in epileptic hippocampi, while NKCC1 expression was downregulated exclusively by the single high dose injection of BDNF. Development of spontaneous recurrent seizures was delayed but not prevented by the treatment, and hyperexcitability behaviors were ameliorated for a short period of time. To prevent GABA-A mediated depolarization and design appropriate treatment strategies for temporal lobe epilepsy, chloride transporters can be considered as a target. Future studies are warranted to investigate any possible therapeutic effects of BDNF *via* altering chloride transporters expression.

Key words: temporal lobe epilepsy, BDNF, GABA, KCC2, NKCC1

## INTRODUCTION

Temporal Lobe Epilepsy (TLE), the most common form of human partial epilepsy (Engel 2001), is poorly controlled by current antiepileptic pharmacotherapy (Cohen et al. 2003). Furthermore, despite a large body of experimental work, TLE epileptogenesis is still poorly understood. However, it has been shown that changes in GABAergic signaling play an important role in this regard (Cohen et al. 2003). Gamma-Aminobutyric Acid (GABA), the main inhibitory neurotransmitter in the adult central nervous system (CNS), at early developmental stages depolarizes target cells through an outwardly directed flux of chloride. In mature neurons, because of the low level of intracellular chloride ($[Cl^-]_i$), GABA triggers membrane hyperpolarization due to passive influx of chloride down its electrochemical gradient. In contrast, immature neurons have an elevated intracellular $[Cl^-]_i$, so that GABA triggers chloride efflux and membrane depolarization (Ben-Ari 2002). Two cation-chloride cotransporters may be especially important in controlling neuronal

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$[Cl^-]_i$: the Na-K-2Cl cotransporter (NKCC1) loads neurons with $Cl^-$ and favors depolarizing responses to GABA, whereas the K-Cl cotransporter (KCC2) normally extrudes $Cl^-$ ions, promoting a hyperpolarizing response (Ben-Ari et al. 2007). In the adult brain, under pathophysiological conditions such as epilepsy and trauma, alterations in the balance of NKCC1 and KCC2 activity may determine the switch from a hyperpolarizing to a depolarizing effect of GABA (Payne et al. 2003). Various lines of evidence correlate epileptogenesis with altered functional expression of NKCC1 and KCC2 transporters. Woo et al. demonstrated that animals deficient in KCC2 exhibit frequent generalized seizures and die shortly after birth (Woo et al. 2002). Okabe and coauthors (2002) observed activity-dependent increases in the mRNA of NKCC1, in the piriform cortex in a rat amygdala-kindling model. In addition, quantitative RT-PCR analyses of surgical specimens taken from the subiculum of patients with drug-resistant temporal lobe epilepsy revealed upregulation of NKCC1 mRNA and down-regulation of KCC2 (Palma et al. 2006). Furthermore, in a study by Huberfeld and colleagues (2007) on biopsies from human epileptic tissue, perturbed chloride homeostasis and GABAergic signaling was demonstrated, and Bumetanide at doses that selectively block NKCC1, suppressed interictal activity.

To design appropriate treatment strategies for these types of epilepsies, KCC2 and NKCC1 transporters should be considered as a target (Payne et al. 2003). Brain-derived neurotrophic factor (BDNF) is one of the underlying players in the regulation of KCC2 expression. Aguado and coworkers (2003) indicated that BDNF acting *via* tyrosine kinase B receptor (TrkB) can increase KCC2 expression during early development in embryos, and in a recent study, Mao and others (2011) showed a novel BDNF/TrkB signaling pathway that profoundly affects neuronal chloride homeostasis. On the other hand, it has been shown by Fukuchi and colleagues (2009). that TrkB receptor of BDNF plays an important role in epileptogenesis. In addition, in a study by Rivera and coauthors (2004) KCC2 expression in mature central neurons was downregulated by BDNF, indicating two modes of BDNF-mediated regulation of KCC2 expression.

Recently, Shulga and others (2008) reported that BDNF increases KCC2 mRNA levels in axotomized central neurons indicating that injured neurons reverse their response to this neurotrophin by switching the BDNF-induced downregulation of KCC2 to upregulation.

Considering the altered functional expression of KCC2/NKCC1 transporters in temporal lobe epilepsy and the role of BDNF in the regulation of KCC2 expression during development and CNS injury we assessed the possible contribution of BDNF on KCC2/NKCC1 expression, ictogenesis and behavioral alterations in a rat model of temporal lobe epilepsy.

## METHODS

### Animals

Adult male Wistar rats weighing 250–270 g were purchased from Tehran Pasteur Institute and housed in a controlled environment (07:00 AM/07:00 PM light/dark cycle; 22±1°C), two weeks before the start of experiments. They had free access to food and water. All experiments were performed in accordance with the Helsinki declaration, and the experiments were approved by the ethical committee of Iran University of Medical Sciences (#1090).

### Pilocarpine-induced Epilepsy

Pilocarpine hydrochloride (Sigma, 350 mg/kg), a muscarinic cholinergic agonist, was administered intraperitonealy (ip) to induce Status Epilepticus (SE). Animals were pretreated with the cholinergic antagonist scopolamine methyl nitrate (Sigma, 1 mg/kg, ip) 30 minutes before the pilocarpine injection to reduce peripheral cholinergic effects (Ferhat et al. 2003). The animal's behavior was observed for several hours thereafter, and scored according to Racine's classification (Racine 1972). Only rats that displayed SE (stages 3–5) for 3–4 hours were selected, and this period of robust seizures was terminated by a single injection of diazepam (7 mg/kg, ip). Animals were hand fed after SE until they could eat and drink on their own. Age-matched naive rats or animals receiving a saline injection instead of pilocarpine were used as controls. Since there was no difference between control groups, data from both groups were pooled (Table I).

### Monitoring of animals for spontaneous recurrent seizures

Two weeks after pilocarpine injection, all rats were video monitored for 12 weeks (8 hours/day, 5 days/week) to record spontaneous seizures.

Table I

| Animal Groups tested in this study | | | | | | |
|---|---|---|---|---|---|---|
| | | | Number of animals and post-SE survival time | | | |
| | | | Biochemistry[1] | | Behavior and chronic seizure[2] | |
| Groups | Seizure Condition | BDNF treatment | Number | Post-SE survival | Number | Post-SE survival |
| Control[3] (naive) | No | No | 5 | Not applicable | 6 | Not applicable |
| Seizure model | Pilocarpine | No | 7 | 1wk (*n*=2)[4]<br>2wk (*n*=2)[5]<br>2wk (*n*=3)[5,6] | 6 | 12 wk |
| Sham[3] | Saline (pilocarpine solvent) | No | 3 | Not applicable | 6 | Not applicable |
| Treatment multiple injections | Pilocarpine | Multiple BDNF injections | 3 | 2 wk | 6 | 12 wk |
| Treatment single injection | Pilocarpine | Single BDNF injection | 3 | 2 wk | 6 | 12 wk |
| Control for treatment multiple injections[6] | Pilocarpine | Multiple PBS (BDNF solvent) injections | 3 | 2 wk | 6 | 12 wk |
| Control for treatment single injection[6] | Pilocarpine | Single PBS (BDNF solvent) injection | 3 | 2 wk | 6 | 12 wk |

[1]Right and Left hippocampi of the same animals were used for Western-Blot and Real Time RT-PCR tests, respectively; [2]Six animals from each group underwent video monitoring for spontaneous seizure detection as well as post-SE hyperexcitability tests, which were performed from 2nd week to 12th weeks post-SE; [3]Their data were pooled together, because there was no difference between them; [4]To evaluate changes in KCC2 expression after SE, we decapitated pilocarpine treated animals at two time points (7th day and 14th day) after SE (Fig. 1); [5] Five pilocarpine treated animals were decapitated 14 days after SE, *n*=2 were used to evaluate changes in KCC2 expression after SE (Fig. 1) and *n*=3 were used to evaluated effect of BDNF treatment (Figs 2–4); [6]Their data were pooled together, because there was no difference between them.

**stereotaxic surgery**

Five days before the pilocarpine injection, animals of treatment groups were placed in a stereotaxic frame, under ketamine (87 mg/kg ip) and xylazine (13 mg/kg ip) anesthesia. A guide cannulae was implanted in the dorsal hippocampus (coordinates: 1.5 mm lateral and 1.7 mm posterior to bregma, 3.0 mm deep from dura) according to Pellegrino and Cushman atlas (1979).

**BDNF injection *in vivo***

The treatment group was divided into two sub-groups (*n*=9 each): One group received multiple

BDNF microinjections (Sigma, Concentration: 1 μg/μl, Injection volume: 2.5 μl, was injected 4 times at days 10, 11, 12, 13 after status epileticus), while the other group was administered with a single high-dose of BDNF (Sigma, Concentration: 1 μg/μl, Injection volume: 10 μl, injected at day 13 after status epileticus). Age-matched epileptic rats received the same amount of BDNF solvent, Phosphate Buffered Saline (PBS), as control. All of the injections were bilateral. Since there was no difference between these controls and the model group (epileptic rats without treatment), their data were pooled together (Table I).

**Behavioral evaluation**

One week after status epilepticus, 6 rats from each group were evaluated in different hyperexcitability tests. All tests were performed twice weekly for 12 weeks. According to previous studies, pilocarpine-treated epileptic animals are more easily agitated compared to normal animals (Rice et al. 1998). A post-seizural Behavior Battery (PSBB) of tests described by Rice and coauthors (1998) was used to discriminate hyperexcitability differences between groups.

Approach-response test

A pen held vertically is moved slowly toward the face of the animal. Responses were recorded as 1 – no reaction; 2 – the rat sniffs at the object; 3 – the rat moves away from the object; 4 – the rat freezes; 5 –the rat jumps away from the object; and 6 – the rat jumps at or attacks the object.

Touch-response test

The animal is gently prodded in the rump with the blunt end of a pen. Responses were recorded as 1 – no reaction; 2 – the rat turns toward the object; 3 – the rat moves away from the object; 4 – the rat freezes; 5 – the rat turns toward the touch; 6-the rat turns away from the touch; and 7 – the rat jumps with or without vocalizations.

Finger-snap test

A finger snap several inches above the head of the animal is performed. Responses were recorded as 1 – no reaction; 2 – the rat jumps slightly (normal reaction); and 3 – the rat jumps dramatically.

Pick-up test

The animal is picked up by grasping it around the body. Responses were recorded as 1 – very easy; 2 – easy with vocalizations; 3 – some difficulty, the rat rears and faces the hand; 4 – the rat freezes; 5 – difficult, the rat avoids the hand by moving away; and 6 – very difficult, the rat behaves defensively, and may attack the hand.

These tests were done by three independent observers; the means of their scores were calculated for each animal for each test. The tests were performed in the home cage with 30 minutes interval between each test.

**Western blot**

Western blotting was performed on hippocampal extracts from different groups. For detecting KCC2 and NKCC1 proteins separately, hippocampal tissue

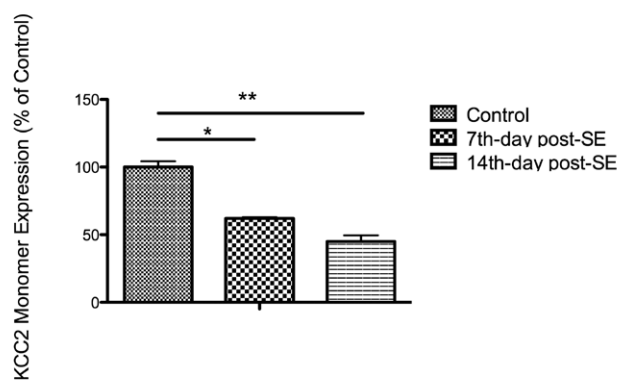


Fig. 1. Gradual down-regulation of KCC2 protein after pilocarpine induced status epilepticus (SE): KCC2 protein expression was analyzed in post-mitochondrial supernatant obtained from whole hippocampal homogenates of control, 1-week post-SE and 2-week post-SE animals. Expression of KCC2 monomer (130–140 kDa) was normalized to the beta-actin (KCC2/Actin) and then plotted as percentage of control protein levels in the histogram comparing groups. Each column represents mean ± SEM. Western blot analysis showed that KCC2 protein expression is decreased significantly on days 7th and 14th post-SE. (*$P$<0.05, **$P$<0.01 by one-way ANOVA with *post-hoc* Tukey Test) [$n$=2 per group, 2 western blot experiments per animal (Means from the two experiments were taken as a result for one animal)].

was homogenized and immediately divided into two equal-volume aliquots. Briefly, the samples were homogenized in Radioimmunoprecipitation Assay (RIPA) buffer [150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS (sodium dodecyl sulphate), 50 mM Tris-HCl pH 7.5], supplemented with a protease inhib-

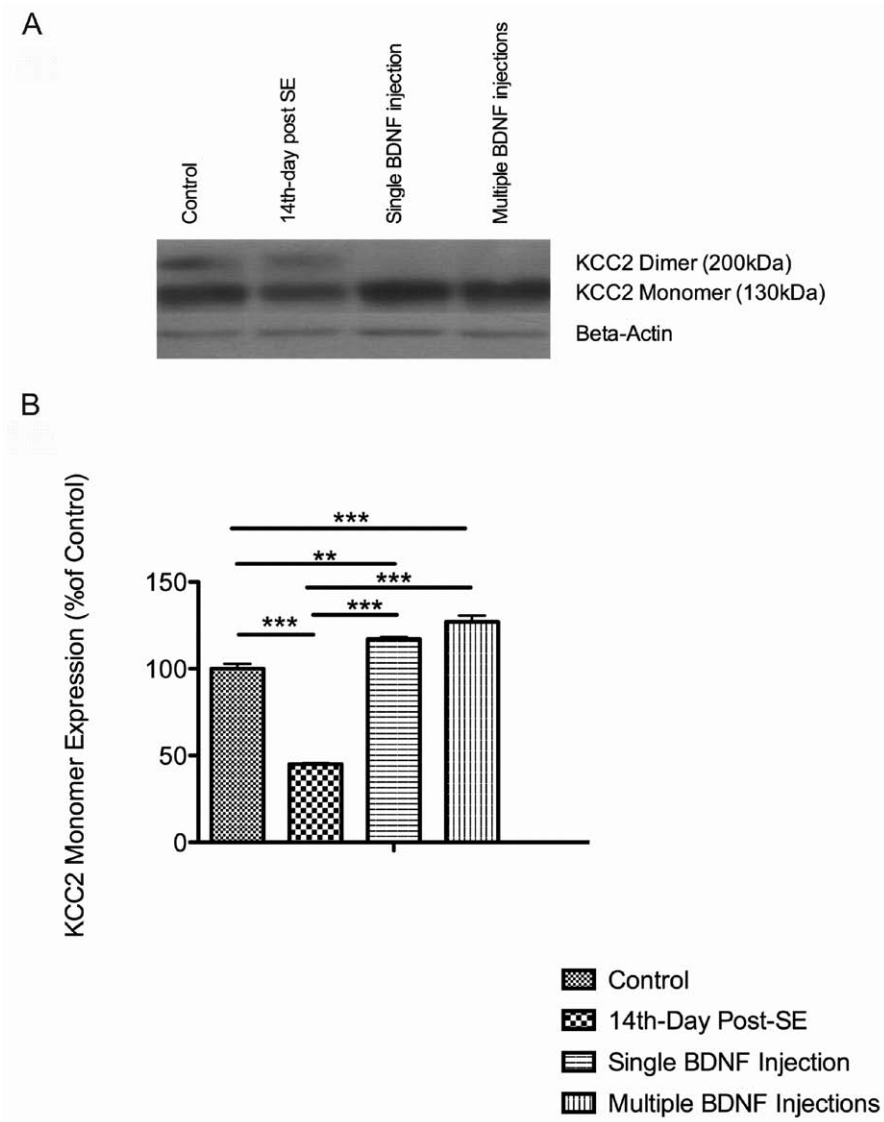


Fig. 2. (A) Representative image of KCC2 western blot for the 4 groups. KCC2 protein expression was analyzed in post-mitochondrial supernatant obtained from whole hippocampal homogenates of different groups, and it was shown that monomeric KCC2 levels were rescued by BDNF in hippocampi after temporal lobe epilepsy, but the bands representing KCC2 dimers were below the level of detection in both treated groups. The 130–140 kDa and the >200 kDa bands correspond to the monomeric and oligomeric proteins, respectively. Beta-actin (~42 kDa) was used as a control. (B) Quantification of KCC2 expression in epileptic rats normalized to controls. The expression of KCC2 was normalized to beta-actin (KCC2/Actin) and then plotted as percentage of control comparing groups. Each column represents mean ± SEM. BDNF induced a significant increase in monomeric KCC2 levels when administered either as a single or multiple injections compared to epileptic untreated controls 14 days post-SE. Treating with BDNF resulted in higher KCC2 levels than in control animals (**$P$<0.01, ***$P$<0.001 by one-way ANOVA with *post-hoc* Tukey Test) ($n$=3 per group, and 2 experiments per animal). Groups: control: control rats; 14th day post-SE: post-SE rats that did not receive BDNF, and were sacrificed at 14th day post-SE; single high dose BDNF injection: post-SE rats that received 10 μg BDNF as a single dose, and were sacrificed at 14th day post-SE; multiple BDNF injections: post-SE rats that received four times 2.5 μg of BDNF injections, and were sacrificed at 14th day post-SE.

itor cocktail (Pierce), then centrifuged at 14 000 g for 20 minutes at 4°C. The supernatant was transferred to a new tube and protein concentration was determined by the Bradford protein assay kit (Pierce). Equal amounts of protein samples (KCC2: 30 mg, NKCC1: 100 mg) and pre-stained protein ladder (Pierce) were electrophoretically (Bio-Rad) separated on 8% SDS polyacrylamide gels, and transferred to a Polyvinylidene Difluoride (PVDF) membrane. The blots were incubated in 5% skimmed milk in Tris-Buffer Saline Tween20 (TBST) for 1 hour at room temperature, and then incubated overnight with a primary antibody (abcam, Rabbit polyclonal anti-KCC2 and anti-NKCC1 antibodies 1/1 000 and 1/4 000, respectively) at 4°C. The blots were rinsed with TBST three times and incubated with horseradish peroxidase-conjugated goat polyclonal secondary antibody to rabbit Ig for 1 hour. After washing with TBST, protein bands were visualized using the enhanced chemiluminescence assay (ECL, Amersham). Quantitative measurement of KCC2 and NKCC1 protein bands was performed with UVIdoc software, and beta-actin was used as control.

### Real-time reverse transcription polymerase chain reaction

Real-time RT PCR was performed on hippocampal extracts from different groups ($n$=3 for each group). Briefly, total cellular mRNA was isolated using QIAzol (Qiagen) according to the manufacturer's protocol, which subsequently underwent cDNA synthesis by reverse transcriptase (Vivantis Cat No: RTPL12). HPRT1 (Hypoxanthine Phosphoribosyltransferase 1) was utilized as housekeeping gene. Specific primers for rat KCC2 were purchased from Qiagen. Gene expression levels were measured by Rotor Gene 6 000 (Corbett, Concorde, NSW, Australia). We calculated relative expression ratio using the Relative Expression Software Tool 2009 (REST).

### Statistical analyses

For the Western blot experiments, statistical comparisons among the groups were performed by one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey HSD for individual differences. The significance of differences in behavioral parameters within each group was analyzed by using repeated-measures ANOVA. Multiple comparison of post hoc analysis was considered using Bonferroni adjustment method. The level of significance was set at $P$<0.05. Relative expression software tool (REST) was used for statistical analysis of relative expression results in real-time PCR (Pfaffl et al. 2002)

## RESULTS

### KCC2 protein levels decrease after Status Epilepticus

To follow the time course of KCC2 changes after SE, we used Western blot analysis to compare KCC2 levels at two time points post-SE. KCC2 levels were significantly decreased at one and two weeks post-SE to 62% ($P$<0.05) and 45% ($P$<0.01) of control, respectively (Fig. 1).

### Effect of BDNF treatment on KCC2 expression

BDNF regulates expression of KCC2 through its TrkB receptor (Ben-Ari et al. 2007). We administered BDNF to the dorsal hippocampus (single dose: 10 µg,

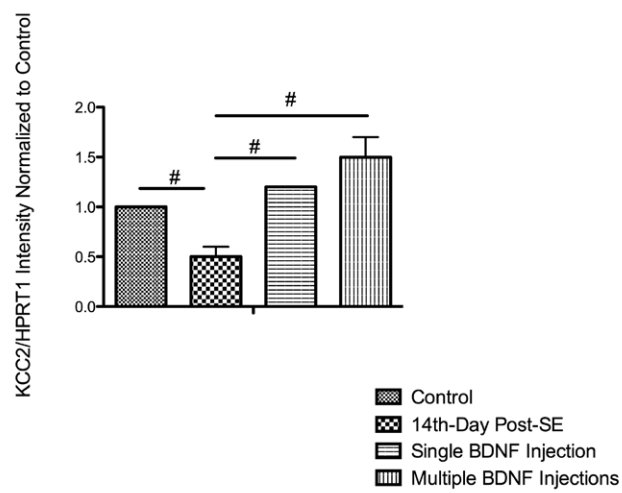


Fig. 3. BDNF treatment rescues KCC2 mRNA expression. Values of KCC2 mRNA are presented as average changes in KCC2 mRNA normalized to average control values. # indicates significance according to REST 2009 software. Groups ($n$=3 per group): control: control rats; 14th day post-SE: post-SE rats that did not receive BDNF, and were sacrificed at 14th day post-SE; single high dose BDNF injection: post-SE rats that received 10 µg BDNF as a single dose, and were sacrificed at 14th day post-SE; multiple BDNF injections: post-SE rats that received four times 2.5 µg of BDNF injections, and were sacrificed at 14th day post-SE.

or four doses: 2.5 μg each) during latent phase of pilocarpine-induced temporal lobe epilepsy. Three animals in each group were decapitated 14 days post-SE, and Western blotting and real-time RT PCR quantified KCC2 expression.

KCC2 Protein Expression

We found that in both treated groups, expression of KCC2 monomeric bands increased markedly ($P$<0.001) compared to untreated epileptic animals. BDNF treatment resulted in higher KCC2 levels than in control animals (Fig. 2). Interestingly, after both single and multiple injections of BDNF the bands representing KCC2 dimers were below the level of detection, indicating that a profound decrease of this oligomeric form occurred.

KCC2 mRNA Expression

KCC2 mRNA underwent a significant decrease in pilocarpine-treated animals compared to controls. Both single high dose and multiple injections of BDNF rescued KCC2 mRNA levels, which, after multiple

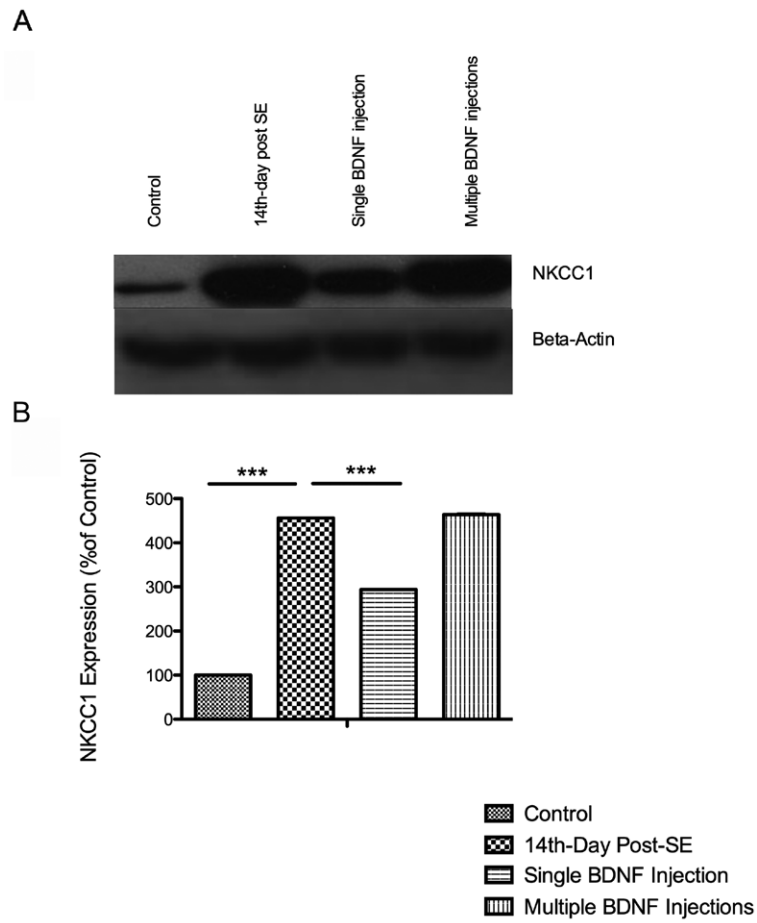


Fig. 4. (A) Representative image of NKCC1 western blot for the 4 groups. NKCC1 protein expression was analyzed in post-mitochondrial supernatant obtained from whole hippocampal homogenates of different groups, and β-actin was used as a control. (B) Quantification of NKCC1 expression in epileptic rats normalized to controls. The expression of NKCC1 was normalized to beta-actin (NKCC1/Actin) and then plotted as percentage of control. NKCC1 protein expression was significantly higher the 2-week post-SE animals than normal controls which was resuced by single high dose injection of BDNF. (***$P$<0.001 by one-way ANOVA with *post-hoc* Tukey Test) ($n$=3 per group, and 2 experiments per animal). Groups: control: control rats; 14th day post-SE: post-SE rats that did not receive BDNF, and were sacrificed at 14th day post-SE; single high dose BDNF injection: post-SE rats that received 10 μg BDNF as a single dose, and were sacrificed at 14th day post-SE; multiple BDNF injections: post-SE rats that received four times 2.5 μg of BDNF injections, and were sacrificed at 14th day post-SE.

BDNF injections, were significantly higher than in control animals (Fig. 3).

### Contribution of BDNF to NKCC1 expression

NKCC1 expression in the hippocampus of the animals from different groups was compared on the 14th day post-SE *via* Western-blotting ($n$=3 each). We observed a significant upregulation of NKCC1 protein levels in epileptic rats at this time point compared to control animals ($P$<0.001). Single injection of a high dose of BDNF resulted in a significant decrease in NKCC1 protein levels compared to the untreated epileptic rats ($P$<0.001), but multiple BDNF injections did not change NKCC1 expression (Fig. 4).

### Behavioral alterations in epileptic rats

According to our general observations, pilocarpine treated rats were more easily startled than control animals, but after BDNF treatment (either single or multiple injection) we were able to handle them comfortably at least for one week.

The PSBB was done at several time points after SE to determine the behavioral changes in epileptic rats and the effect of BDNF treatment in this regard ($n$=6 for each group). When we compared pilocarpine-injected animals with the control group on the 7th day post-SE, a significant difference ($P$<0.05) was observed in the touch-response and pick-up tests, indicating that epileptic rats are more sensitive to environmental

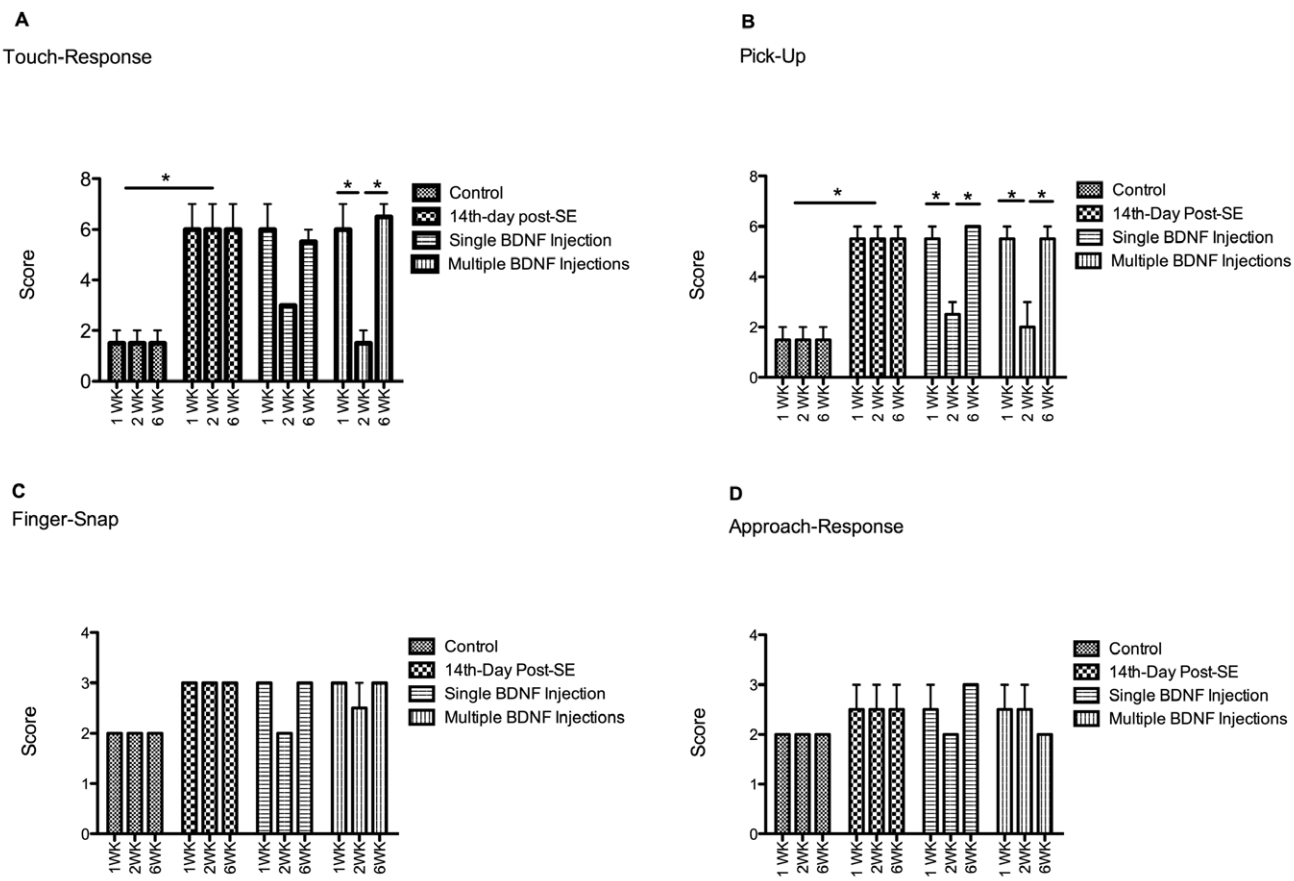


Fig. 5. Post-seizure behavioral changes 1,2 and 6 weeks post-SE: (A) Touch-Response; (B) Pick-up; (C) Finger-Snap; (D) Approach-Response. A significant difference was observed in the touch-response and pick up tests between pilocarpine injected animals and control group at 7th day post-SE (*$P$<0.05, independent sample $t$ test). Within group comparisons indicated that BDNF treatment can ameliorate hyperexcitability behaviors for a short period of time (*$P$<0.017, Bonferroni corrected). Groups ($n$=6 per group): control: control rats; 14th day post-SE: post-SE rats that did not receive BDNF, and were sacrificed at 14th day post-SE; single high dose BDNF injection: post-SE rats that received 10 μg BDNF as a single dose, and were sacrificed at 14th day post-SE; multiple BDNF injections: post-SE rats that received four times 2.5 μg of BDNF injections, and were sacrificed at 14th day post-SE.

stimuli. BDNF treatment counteracted these behaviors on the 14th day post-SE, but this effect lasted only 1 to 2 weeks, and these test scores gradually returned towards baseline (pre-treatment period) (Fig. 5).

### Spontaneous recurrent seizures detection

The earliest spontaneous seizure was observed 2 to 3 weeks post-SE in the untreated epileptic group. All except one of these animals had 1 to 3 spontaneous seizures per week during the video monitoring period. In the two BDNF-treated groups the onset of spontaneous seizures was 4 to 6 weeks post-SE. All the animals of the treated groups had 1 to 3 spontaneous seizures per week, which was not different from the untreated group. These data indicate that BDNF treatment can delay epileptogenesis, but is not sufficient in preventing spontaneous seizure generation ($n$=6 for each group).

## DISCUSSION

Various lines of evidence indicate that BDNF, a member of the neurotrophin family of growth factors (Leibrock et al. 1989), is associated with increased neuronal activity (Tanaka et al. 1997), and has been considered as a proepileptogenic factor (Rivera et al. 2004). On the other hand, according to several reports, the development of epilepsy can be attenuated or postponed by intrahippocampal delivery of BDNF (Koyama and Ikegaya 2005, Paradiso et al. 2009). To address these contrasting effects of BDNF, it is essential to clarify TLE pathogenesis. While no definitive evidence exists to support a single mechanism for temporal lobe epileptogenesis, a large body of evidence suggests that depolarizing GABAergic signaling accompanied by altered expression of the chloride co-transporters KCC2 and NKCC1 contributes to epileptiform activity in animal models of epilepsies as well as human epileptic tissues (Cohen et al. 2002, Dzhala et al. 2005, Palma et al. 2006, Huberfeld et al. 2007, Munoz et al. 2007, Pathak et al. 2007). In this study we showed that there is a notable imbalance between expression of the two chloride transporters in an animal model of TLE. NKCC1 expression was markedly increased, while that of KCC2 was significantly reduced in epileptic hippocampi compared to intact controls. This alteration in the expression of chloride transporters together with a depolarizing, immature-like response of GABA is in line with other pathophysiological conditions such as neuronal trauma, axotomy and spinal cord injury (Nabekura et al. 2002, Payne et al. 2003, Shulga et al. 2008, Boulenguez et al. 2010, Ziemlinska et al. 2014). The depolarizing GABA-A mediated response during development is due to chloride uptake mediated by NKCC1, and the hyperpolarizing response in mature neurons is detectable when KCC2 expression increases causing a $[Cl^-]_i$ reduction (Rivera et al. 1999). Accumulating evidence indicate that upregulation of KCC2 is induced by BDNF leading to the functional maturation of GABA-A mediated responses (Aguado et al. 2003, Carmona et al. 2006, Ludwig et al. 2011). Ludwig and coauthors (2011) demonstrated that BDNF activates expression of the immediate early growth response 4 gene by induction of the TrkB/mitogen activated protein kinase pathway which stimulates KCC2b promoter activity. Furthermore, KCC2 expression is lower in the hippocampus of mice which are deficient in TrkB (Carmona et al. 2006). On the other hand, in adult normal cortical neurons BDNF downregulates KCC2 expression (Rivera et al. 2004) indicating two modes of BDNF-mediated regulation of KCC2 expression: upregulation during development and downregulation in mature central neurons (Shulga et al. 2008). Interestingly, Shulga and colleagues (2008) investigated the role of BDNF on KCC2 expression of axotomized corticospinal neurons, and demonstrated that injury activates a developmental-like mode of BDNF-mediated effects inducing a switch in the effect of BDNF on KCC2 expression from downregulatory to upregulatory. Furthermore, Boulenguez and coworkers (2010) showed that while KCC2 is downregulated after spinal cord injury in rats, intrathecal injection of BDNF (10 μg) can upregulate KCC2 expression 15 days after the injury.

Considering the results of the two recent studies (Shulga et al. 2008, Boulenguez et al. 2010), we investigated the effects of intra-hippocampal injection of BDNF on KCC2 expression during the second post-SE week. Our results showed that application of either a single or multiple doses of BDNF, up-regulated KCC2 expression. While the results we obtained with the single injection of BDNF are in accordance with the findings of Boulenguez and coworkers (2010), in a recent study by Ziemlinska and others (2014) long term BDNF treatment reduced KCC2 expression after a complete transection of the spinal cord.

As shown in Figure 2, during the first 24 hours post BDNF injection, monomeric KCC2 is prevailing while oligomeric KCC2 was below the level of detection. Blaesse and colleagues (2006) showed that age-dependent KCC2 oligomerization occurs throughout the nervous system development, because monomeric KCC2 which is inactive for transport requires oligomerization to be active. Furthermore, they showed that the developmental shift of GABA from depolarizing to hyperpolarizing occurs over several days, and this shift can be determined by both increased gene expression and KCC2 oligomerization. One possible explanation for this finding is that BDNF stimulates KCC2 synthesis and storage, but downregulates its oligomerisation.

Our behavioral studies revealed that hyperexcitability behaviors of animals were ameliorated for a short period of time after BDNF treatment. Furthermore, development of spontaneous recurrent seizures was delayed but not prevented in BDNF-treated animals, suggesting that in this study, an injection of BDNF delayed epileptogenesis. This effect might stabilize for a longer period of time if rats were infused continuously with BDNF using osmotic minipumps. On the other hand, given the disappearance of oligomeric KCC2, these transient behavior improvements might be due to BDNF effects on neurogenesis and embanking neuronal damage (Paradiso et al. 2009).

Another interesting finding of the present study was the reduction of NKCC1 expression by a single high dose of BDNF. To the best of our knowledge, this is the first time there is a direct effect of BDNF on NKCC1 expression. Further studies are warranted to determine why only a high dose injection but not multiple injections of BDNF altered NKCC1 expression.

## CONCLUSION

While recent studies point to NKCC1 inhibition as a promising strategy for TLE treatment (Huberfeld et al. 2007, Brandt et al. 2010, Eftekhari et al. 2013), an increase in KCC2 expression can be considered as an alternative treatment to Bumetanide application. In a recent study, Gagnon and others (2013). showed that enhancing KCC2 activity can be the favored therapeutic strategy to restore inhibition and normal function in pathological conditions involving impaired chloride transport.

If we want to focus on KCC2 activation as a TLE treatment strategy it is essential to find out for how long after induction of TLE, BDNF manages to upregulate KCC2, keeping its immature-state function. On the other hand, as applying exogenous BDNF is not practical and amenable for human patients future experiments are needed to modify KCC2 expression *via* endogenous BDNF. Although, Shulga and coauthors (2008) mentioned in their study that the endogenous levels of BDNF were not enough to upregulate KCC2, more recent studies point to an up-regulation of endogenous BDNF by application of some hormones like tyroxine (Shulga et al. 2009) and statins like simvastatin (Han et al. 2011) in injured neurons. Future studies are warranted to assess any possible therapeutic effects of such agents on temporal lobe epilepsies *via* inducing endogenous BDNF.

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